A STUDY OF pH CHANGES BY MOLDS IN CULTURE MEDIA*†

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It is well-known that the dermatophytes tend to produce an alkaline pH when growing on Sabouraud's medium (Nickerson, O. F. Jillson (1, 2); Peck and collaborators (3, 4); v. Mallinckrodt-Haupt (5, 6); Kadisch (7); Taschdjian (8)). According to Goddard (9), this is due to deamination of amino acids and consequent formation of ammonia. On the other hand, certain non-pathogenic molds, such as Penicillium and Aspergillus species, are known to shift the pH of the medium towards acidity. Physico-chemical peculiarities presumably underlying this effect of saprophytes have been discussed in a monograph by Foster (10). Leise and James utilized this difference in the culture media of dermatophytes and saprophytes for isolating dermatophytes from contaminated material by transplanting them on alkaline media (11, 12); and Littman introduced a special alkaline medium for primary isolation of the dermatophytes (13).

The present investigation deals with some of the aspects of these pH changes associated with the growth of fungi. We are particularly interested in the following points:

1.) The regularity of the phenomenon, i.e. whether changes in pH are produced by all, or only some of the dermatophytes and common laboratory contaminants tested, and whether variations exist in the activity of different strains of one and the same species.

2.) The time relation between the onset of the change in pH and the appearance of visible growth. Early changes in pH, preceding the stage of growth when the organism can be identified as to species, would furnish a valuable indication regarding the pathogenicity or non-pathogenicity of a given culture. (A very early change in pH was observed by MacKee, Herrmann, Karp and Behrendt in subcultures of *M. Audouini* (14, 15).)

3.) The configuration and demarcation of the areas in which the pH changes occur, and the question whether or not these changes produced by growing molds in their media follow the principles applying to changes in acidity within colloidal systems in general. If such were the case, one might consider the possibility that similar processes might occur likewise in the skin *in vivo*, and account for the peculiar configuration of lesions as seen in "ringworm", etc.

Moreover, if the pH changes produced by the cultures are confined to sharply delineated zones—as is characteristic of the distribution of acid and alkali upon diffusion into jellies (see below)—one might expect the particular zone of the medium to contain only growth of the type responsible for the particular change in pH. In that case, dermatophytes should be found exclusively in an area of

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raised-, and contaminants in an area of lowered pH, and isolation of pure cultures would thus be greatly facilitated.

METHOD AND MATERIALS

Standard Petri dishes (9 cm. in diameter, 1 cm. in depth) containing 30 ml. of Sabouraud's medium or conservation medium (3% peptone without glucose) were inoculated at the center or the periphery with various dermatophytes and saprophytes. Pure and mixed cultures were grown.

A specially prepared universal pH indicator* was added to the medium.

Approximately 3 ml. of a 50% dilution of this solution with distilled water were placed on the surface of the culture-bearing medium 24 hours prior to removal of the entire cake for examination of the presence and distribution of acid and alkali.

Complete removal of the intact cake was facilitated by warming the dish on a water bath to 54° C. Two to three minutes later, when the temperature had fallen to 48° C., the plate was turned upside down and the agar mass inside was gently released by tapping on the bottom of the dish.

The volume of the medium used allowed for even imbibition of the indicator and at the same time for sufficient depth in which there was no masking of the indicator color by mycelium and/or pigment formed by the culture. When necessary for undisturbed visualization, it was possible to slice off the culture. Any shade of indicator color in the medium could be observed at the growth-free lower surface.

The pH ranges were estimated by comparison of the color shade in the medium with a standard chart of colors issued for this purpose. The readings were checked from time to time by additional determinations with the Beckman electrometer (Model G), using a "fiber-type reference" (calomel) electrode (Beckman No. 270) and a glass electrode (Beckman No. 290) which was placed on the surface of the medium.

Cultures of the organisms listed in Table I were employed. They were obtained (a) from scrapings of infected skin or nails, or from hairs; (b) from media accidentally contaminated with a saprophyte suitable for our purpose; (c) from stock collections of fungous cultures (*T. Megnini*†; *Aspergillus fumigatus*‡; *Aspergillus clavatus*‡).

RESULTS

a) *Regularity and direction of pH changes*

Table I shows that all of the eleven species of dermatophytes studied changed the pH of the media toward alkalinity. Nearly all of the common contaminants tested produced a pronounced shift towards acidity; only three organisms—one organism of the Aspergillus group (*A. fumigatus*), one of the Candida group, and one of the Penicillium group—failed to change noticeably the a priori acid pH

* The same indicator (Stanscien Indicator—supplied by Standard Scientific Supply Corp., New York, N. Y.—containing 0.1 gram of powdered Parstains Universal Indicator in 75 ml. of methanol (41%) and distilled water (59%), with sufficient sodium hydroxide added to effect solution and a dark green color) was previously employed for pH investigations on the skin surface by Herrmann, Behrendt and Karp (15), Herrmann (16), Behrendt and Green (17), and Bernstein and Herrmann (18).

† We are indebted to Dr. Rhoda Williams Benham, Assistant Professor, Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York, N. Y., for the supply of this organism.

‡ We wish to thank Chas. Pfizer & Co., Brooklyn, N. Y., for kindly supplying us with this organism from their stock culture.

TABLE I

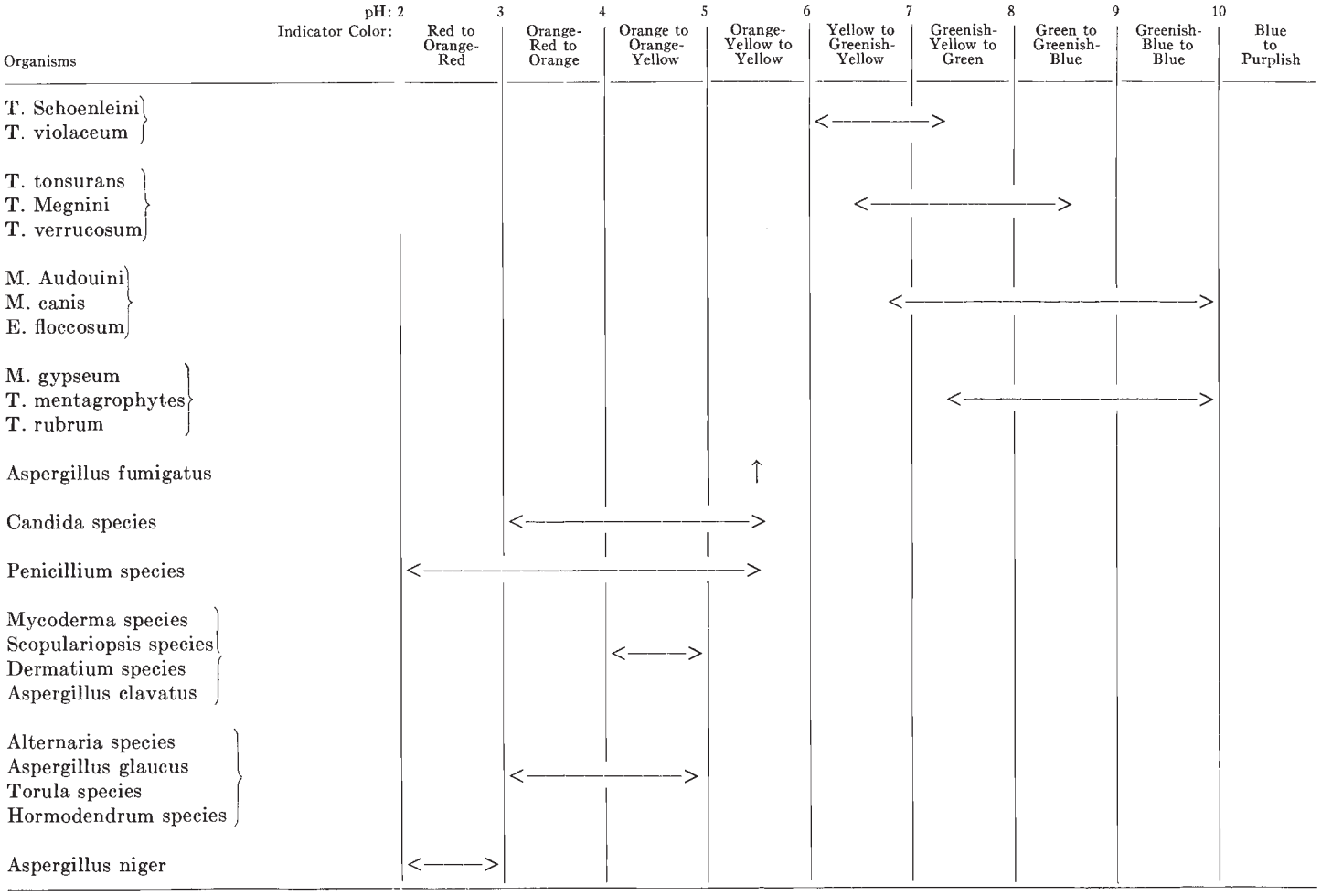
Incidence and direction of pH shifts produced by different organisms in Sabouraud's medium (original pH 5.6)

Organisms	No. of Original Cultures*	Total No. of Cultures (including Subcultures)	Producing pH above 5.6		Producing pH below 5.6	
			No. of Original Cultures*	Total No. of Cultures (including Subcultures)	No. of Original Cultures*	Total No. of Cultures (including Subcultures)
Dermatophytes						
M. Audouini	39	59	39	59	0	0
M. canis	31	37	31	37	0	0
M. gypseum	7	9	7	9	0	0
T. mentagrophytes	18	26	18	26	0	0
T. rubrum	9	12	9	12	0	0
T. tonsurans	9	11	9	11	0	0
E. floccosum	9	16	9	16	0	0
T. violaceum	2	2	2	2	0	0
T. Schoenleini	1	1	1	1	0	0
T. verrucosum	1	1	1	1	0	0
T. Megnini	1	2	1	2	0	0
Saprophytes						
Aspergillus niger	43	53	0	0	43	53
Aspergillus glaucus	9	9	0	0	9	9
Aspergillus fumigatus	1	1	0	0	0 (pH unchanged in one)	0 (pH unchanged in one)
Aspergillus clavatus	1	1	0	0	1	1
Candida species	6	8	0	0	5 (pH unchanged in one)	7 (pH unchanged in one)
Penicillium species	31	38	0	0	30 (pH unchanged in one)	37 (pH unchanged in one)
Torula species	3	3	0	0	3	3
Dermatium species	3	3	0	0	3	3
Alternaria species	8	10	0	0	8	10
Hormodendrum species	3	3	0	0	3	3
Mycoderma species	1	1	0	0	1	1
Scopulariopsis species	1	1	0	0	1	1

* The "original" dermatophyte cultures were obtained from different patients each, while the "original" saprophyte cultures were obtained by inoculation from different cultures or by inadvertent contamination.

GRAPH I

Ranges of acidity produced by different species of molds in Sabouraud's medium



of the medium. Hence, any noticeable change in pH produced by a contaminant in our series was in the direction of acidification.

Differing *degrees* in the changes produced were obtained with different species of both groups. Only minor variations were observed with different strains of one and the same species. The approximate pH ranges obtained with different species are shown in Graph I.

b) *Time of pH changes in relation to phase of culture growth*

In the case of the dermatophytes, the shift towards alkalinity occurred shortly after the beginning of visible growth. Some of the saprophytes, however, acidified the medium even prior to the appearance of growth. Acidification preceding visible growth by 12–24 hours was noticeable in particular in the culture media of Penicillium and Aspergillus.

The colored zones around the growing colonies advanced rapidly—and within 2 to 3 weeks after the beginning of growth the entire medium had changed color.

The rate of the shifts in pH was greatest during the early stages of growth and decreased gradually in later stages. Nevertheless, the shifts, as indicated by continued changes in color, progressed as long as fungous growth was observed to continue, i.e. for periods of 2 weeks to 6 months.

c) *Configuration of zones of altered pH in media of pure cultures*

During the early phases of growth, the cultures of the dermatophytes showed a zone of intense alkalinization 2–4 mm. in width immediately adjacent to the colony and surrounded by a zone of lesser alkalinization. Analogous zones of stronger and weaker acidification were produced in the medium by most of the saprophytes studied, most distinctly so by Aspergillus and Penicillium. These concentric zones of stronger and weaker acidity, respectively, remained sharply defined. In other words, no gradients in color intensity were noted between them. The concentric zones were visualized most clearly in transmitted light. Their pattern persisted in the medium after the colonies were removed.

On "conservation agar", with an initial pH of 7, containing 3% peptone and no glucose, the dermatophytes produced a considerably higher degree of alkalinity than on Sabouraud's medium (initial pH: 5.4–5.6). The saprophytes grew poorly on this medium and produced only weak acidification.

d) *Configuration of zones of altered pH in media of mixed cultures*

In mixed cultures of dermatophytes and deliberately grown saprophytes or in mixed cultures of dermatophytes and accidental contaminant fungi (Sabouraud's medium), every dermatophyte colony produced a sharply demarcated area of alkalinization, and every saprophyte or contaminant colony an equally distinct area of acidification (Fig. 1). As the distance between the outer margins of the two respective colonies decreased, and the pH shifts in both directions became more marked, the areas became separated by a sharply delineated, narrow, canary-yellow "band of neutrality". For some period of time, this "band" widened while the pH shifts on either side increased in either of the respective directions.

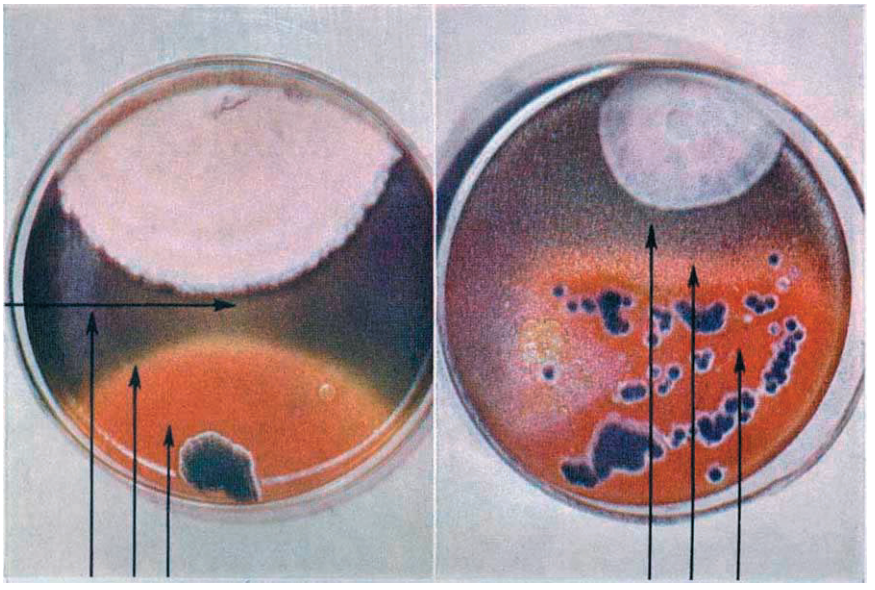


FIG. 1 FIG. 2

FIG. 1. Culture of Microsporon gypseum (top) and of Aspergillus niger (Sabouraud's medium). Aspergillus niger inoculated 10 days after inoculation of Microsporon gypseum. Incorporation of Universal pH indicator in medium—10 days after inoculation of Aspergillus niger—reveals a) zone of alkalinization; b) "neutral band"; c) zone of acidification. Light stripe (p) at edge of Microsporon gypsium culture is effect of brownish-yellow pigment produced by organism.

FIG. 2. Growth of Microsporon Audouini (top) and multiple colonies of Aspergillus niger (Sabouraud's medium). Aspergillus niger inoculated 10 days after inoculation of Microsporon Audouini. Incorporation of Universal pH indicator in medium—five days after inoculation of Aspergillus niger—reveals a) zone of alkalinization; b) "neutral band"; c) areas of acidification.

Due to the usually more rapid growth of the saprophyte, and the consequently more rapid production of acid, the zone of alkalinization around the dermatophyte colony gradually narrowed. The neutral zone separating the 2 areas was pushed toward the colony of the pathogen and at the same time diminished in width as the acid zone surrounding the saprophyte colony expanded. The dermatophyte finally ceased to grow altogether.

Because of the differences in the rates of growth of the two groups of organisms tested, the color contrasts in the medium were most apparent when the dermatophyte was allowed to grow for 10 days prior to inoculation of the same plate with the saprophyte, and when the inocula were placed opposite each other at the periphery of the Petri dish (Figs. 1 and 2).

e) *Early identification of type of fungous growth by means of pH indicator*

Addition of the indicator to more than 30 young cultures too small for gross identification, made it possible to recognize a given culture as pathogenic or non-pathogenic. Similarly, the marked pH changes in young mixed cultures permitted early differentiation between the grossly similar dermatophyte and saprophyte colonies. *The characteristic sharp delineation of the areas of differing pH allowed early isolation* by transfer of the dermatophyte colonies, before they became overgrown with the contaminant(s) with which they were growing side by side (Fig. 2).

DISCUSSION

Our results furnish further evidence that, despite some individual variations in either group, the dermatophytes quite regularly change the pH of Sabouraud's medium toward alkalinity, and the saprophytes, as a rule, towards acidity (Table I, Graph I).

It appears noteworthy that—contrary to the observations of v. Mallinckrodt-Haupt (6)—the pathogens producing a shift toward alkalinity included all of 9 strains of Epidermophyton floccosum employed in this study.

The general difference observed throws an interesting sidelight on fundamental metabolic differences between the 2 groups of organisms. The keratophilic dermatophytes apparently metabolize chiefly protein materials, while the saprophytes seem to utilize essentially glucose and to produce acids by its breakdown. This is implied by the behavior of the 2 groups of organisms on the glucose-free conservation agar.

The fact that these pH changes permit early differentiation between a pathogenic and a non-pathogenic mold in contaminated cultures is of practical importance, since isolation of the pathogen in pure culture and its subsequent identification are thus facilitated.

The sharp delineation of the areas with contrasting ranges in acidity is a characteristic result of diffusion of acid and/or alkali into colloidal systems (19). The configuration of these zones and their biologic significance was studied and described in detail by Liesegang (19, 20, 21).

Instead of a gradient in pH between two adjacent zones, there is a sharp

borderline of electric equilibrium separating two opposite ranges of acidity. On the alkaline, electronegative side of the border, hydroxyl ions accumulate, whereas an equivalent number of positively charged particles "line up" on the acid, electropositive side. The equilibrated barrier (electric "double membrane") prevents particles of either charge from passing to the opposite side across this "front line of defense" (19, 20, 21).

The equilibrated border zone then tends to increase in width, forming a well-demarcated "neutral" zone between the two areas, whether the electrolytes responsible for the changes in pH are derived from the metabolizing activity of the growth of different living organisms, or from extraneous addition of acid and base to the medium.

In colloidal systems, zones of differing degrees in acidity or alkalinity may arrange themselves in the form of concentric bands or rings ("Liesegang rings" (19)); and the concentric zones of differing degrees of acidification or alkalinization, which we observed surrounding our fungous colonies, may be interpreted as Liesegang rings. Liesegang himself (20, 21), as well as various other investigators (22), related the concentric growth of fungous cultures to the "rhythmic" distribution of agents participating in chemical reactions within colloidal systems.

SUMMARY

1. A universal pH indicator was added to culture media of various molds, including several species of dermatophytes (skin pathogens) and several species of saprophytes. In the media of cultures obtained from different strains of eleven species of dermatophytes, a distinct shift in pH toward alkalinity was observed at, and around, the site of growth. In the media of different "common laboratory contaminants", a distinct shift toward acidity was observed in nearly all instances at, and around, the site of the growth; only occasionally was no change in pH discernible in the *a priori* acid medium. Characteristic differences in degree of the shifts in pH were obtained with different species in both groups.

2. The pH changes in the medium were apparent shortly after inception of visible growth in the media of dermatophytes, whereas they sometimes even preceded the appearance of visible growth of saprophytes. These pH changes permitted early differentiation between pathogenic and non-pathogenic molds and facilitated the selection, transplantation, and isolation of the pathogens from contaminated cultures.

3. The zones of alkalinization or acidification were sharply delineated. In mixed cultures of dermatophytes and saprophytes, the areas of opposite ranges in pH remained clearly separated. At the meeting points of the two areas, a common borderline formed, on one side of which the medium was alkaline, and on the other acid; subsequently, a sharply delineated band of near neutral pH developed—and for some time widened—between the two zones. The configurations and delineations observed are due to the formation of electric border equilibria which are known to result from the diffusion of alkaline and acid electrolytes through gelatinous substances.

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